Quantitative Evaluations of the Effect of UV Irradiation on the Infectivity of HTLV-III (AIDS Virus) with HTLV-I-Carrying Cell Line, MT-4

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The effect of UV irradiation on HTLV-III was quantitatively studied to evaluate the dosage of UV irradiation which inactivates the virus for sterilization of blood products and for laboratory decontamination. In order to estimate the biologic activity and quantitation of the virus, induction of HTLV-III-specific antigens and inhibition of DNA synthesis in MT-4 cells infected by UV-irradiated HTLV-III were detected by indirect immunofluorescence technique and proliferation assay using [^{3}H]thymidine uptake, respectively. Furthermore, plaque-forming assay was performed to count the infectious viral particles. Results showed that HTLV-III was completely inactivated by 5000 J/m^2 UV irradiation. Cloned UV-irradiated HTLV-III (UV-1) was obtained from a plaque that was formed by 2000 J/m^2 UV-irradiated virus. When MT-4 cells were infected by the clone UV-1, ballooning degeneration of cells was predominantly induced. These ballooning cells were not usually observed in MT-4 cells infected by unirradiated HTLV-III. The resistance to UV was not different between clone UV-1 and unirradiated HTLV-III. *J Invest Dermatol 87:239–243, 1986*

The etiologic agent of the acquired immune deficiency syndrome (AIDS) is a newly recognized retrovirus called human T-lymphotropic virus type III (HTLV-III) [1], lymphadenopathy-associated virus (LAV) [2], or AIDS-associated retrovirus (ARV) [3]. The virus is frequently isolated from patients with AIDS or AIDS-related complex [4]. The prevalence of antibodies is high in those infected or at high risk, but low in healthy controls [5–8]. Persons with hemophilia are thought to be at high risk for the development of AIDS, since antibodies to HTLV-III/LAV have been found in the majority of these persons who had received clotting factor concentrates [9–11]. It is urgent to discover the means which will directly inactivate the HTLV-III/LAV in blood products before they are administered to patients. Many compounds are now being investigated for their direct effects on the virus itself [12–14]. In attempting to evaluate the effects, however, the lack of quantitative assay systems was the main problem in accurately assessing the amount of the biologically active virus.

Previously, we reported that HTLV-III/LAV efficiently infects and propagates in a large number of HTLV-I, most probably a causative agent of adult T-cell leukemia-carrying cell lines [15], such as MT-4 [16]. As a result, infected cells showed remarkable cytopathic effect (CPE) leading to the inability of further passage of the cells [17]. Subsequently, we also developed quantitative assay systems for HTLV-III, using MT-4 cells [18], such as the plaque-induction and proliferation assay. In the present study, we attempted to evaluate the effect of UV irradiation on the infectivity of HTLV-III by using these accurate assay systems. Our quantitative studies revealed that UV irradiation inactivated the infectivity of HTLV-III at a dosage of 5000 J/m^2.

MATERIALS AND METHODS

Virus Preparation and Treatment of the Virus by UV Irradiation For viral preparation, Molt-4/HTLV-III cell suspension was cultured for 4 days at 37°C in a CO_2 incubator. After spinning down the cells at 400 *g* for 10 min, supernatant was filtrated through a 0.22-μm Millipore membrane. One milliliter of viral solution in uncovered 35-mm plastic dishes in an ice bath were irradiated by a UV lamp (Toshiba, Tokyo, Japan) at dosages of 100, 200, 500, 1000, 2000, and 5000 J/m^2. Then, the viral solution was diluted to one-tenth with fresh medium [RPMI 1640 supplemented with 10% decomplemented fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin]. The intensity of the UV lamp was measured by a UV intensity meter with a filter for 254 nm (Ultraviolet Products Inc., San Gabriel, California) before experiments were started.

Immunofluorescence (IF) Method MT-4 cells were adjusted to a concentration of 1×10^6 cells/ml and 1 ml of the cell suspension was mixed with the same volume of UV-irradiated and unirradiated virus. The MT-4 cells infected by UV-irradiated HTLV-III were incubated at 37°C in a CO_2 incubator. Culture fluid was changed on day 4 after infection. On days 3, 5, and 7 after infection, cells were smeared, dried, and fixed with cold methanol for 3 min. Fixed cells were then incubated with 1:1000 diluted human anti-HTLV-III-positive serum (IF titer; 1:4096) for 30 min at 37°C. Then, the preparation was washed for 15 min with phosphate-buffered saline (PBS). The fluorescein isothio-

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Abbreviations:
AIDS: acquired immune deficiency syndrome
ARV: AIDS-associated retrovirus
CPE: cytopathic effect
FCS: fetal calf serum
HTLV-I: human T-lymphotropic virus type I
HTLV-III: human T-lymphotropic virus type III
IF: immunofluorescence
LAV: lymphadenopathy-associated virus
PBS: phosphate-buffered saline
PLL: poly-L-lysine
RT: reverse transcriptase

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cyanate-conjugated antihuman IgG (Dakopatts A/S, Copenhagen, Denmark) was applied, incubated for 30 min at 37°C, and washed again with PBS. The fluorescent cells were examined under a fluorescent microscope and the percentage of IF-positive cells was calculated.

Proliferation Assay MT-4 cells were adjusted to 1×10^6 cells/ml and a 50-μl aliquot of cell suspension and the same volume of UV-irradiated or unirradiated virus was placed into each well of a 96-well flat-bottom microtiter plate (Corning, New York, New York). On the 3rd day after inoculation of the virus, 100 μl of fresh medium were dropped into each well. The cells were incubated at 37°C in a CO_2 incubator for 5 days with 1 μCi/well of [^{3}H]thymidine (New England Nuclear, Boston, Massachusetts) present for the last 18 h. Cultures were harvested onto glass fiber paper and [^{3}H]thymidine uptake was determined by a liquid scintillation counter. All experiments were carried out in triplicate. Standard deviations of each experiment were less than 5% of the mean cpm.

Plaque-Forming Assay To make an MT-4 cell-monolayer on culture vessels, 1 ml of 50 μg/ml of poly-L-lysine (PLL; M_r 90,000, Sigma Chemical Co., St. Louis, Missouri) were dropped onto 35-mm polystyrene tissue culture dishes (Falcon, Oxnard, California). The dishes were incubated for 1 h at room temperature. Then, PLL-coated dishes were washed 3 times with PBS. One and one-half milliliters of 150×10^4/ml of MT-4 cells were then added into each PLL-coated dish and incubated for 1 h at room temperature. The dishes were gently washed twice with PBS to remove unbound cells. Then, 100 μl of UV-irradiated or unirradiated viral preparation was slowly added to the dishes and incubated for 1 h at room temperature. After adsorption of the virus, 1 ml of the Agarose overlay medium consisting of RPMI 1640 medium with 10% FCS, antibiotics, and 0.6% Agarose (Sea Plaque Agarose, Marine Colloid Corp., Rockland, Maine) was poured into each dish. The dishes were incubated in a CO_2 incubator at 37°C for 3 days and 1 ml of Agarose overlay medium containing neutral red was added. The dishes were incubated at 37°C for 3 more days and visible plaques were counted.

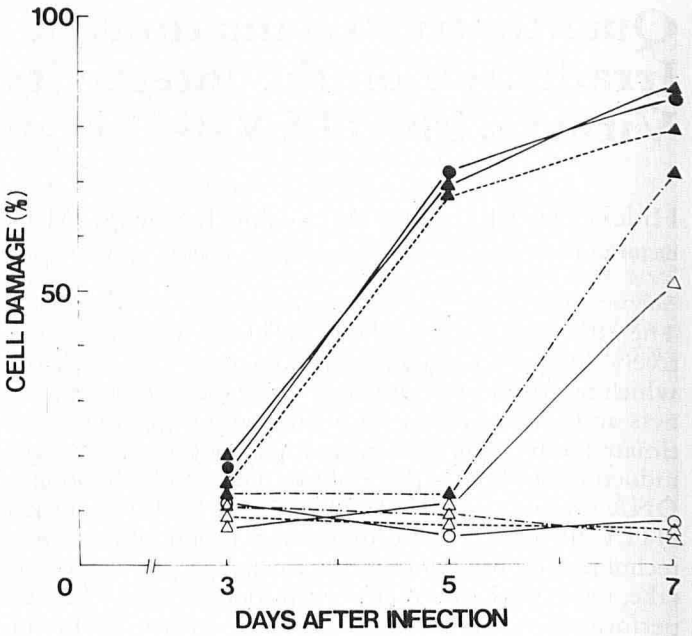

Figure 2. Viral-induced cell damage. On days 3, 5, and 7 after infection, cells were counted in a hemocytometer under microscopy by the trypan blue exclusion method. Control (●——●), 100 J/m^2 (▲——▲), 200 J/m^2 (▲– – –▲), 500 J/m^2 (▲–·–▲), 1000 J/m^2 (△——△), 2000 J/m^2 (△– – –△), and 5000 J/m^2 (△–·–△) UV-irradiated HTLV-III-infected MT-4 and uninfected MT-4 (○——○).

RESULTS

Effects of UV Irradiation on the Virus Infectivity Detected by HTLV-III-Specific Antigen Induction and Cell Damage To evaluate the infectivity of the irradiated virus, expression of HTLV-III-specific antigens was assessed by IF in

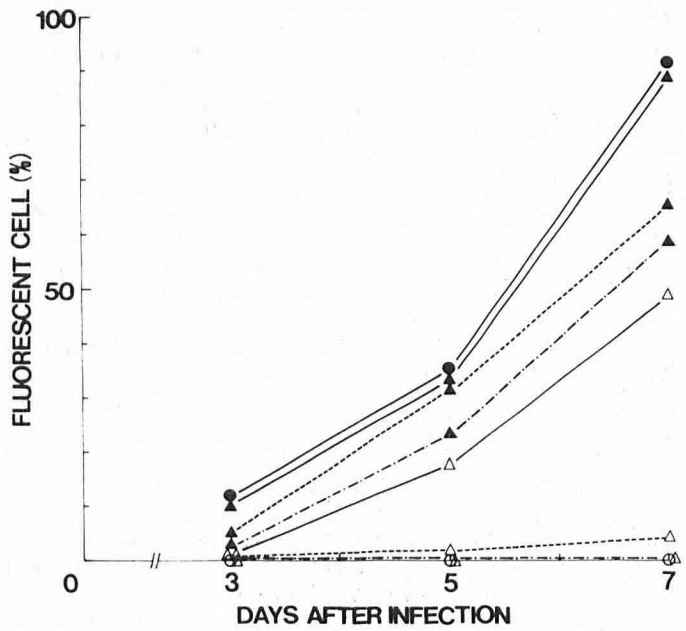

Figure 1. Appearance of virus-specific antigens in UV-irradiated and unirradiated HTLV-III-infected MT-4. More than 500 cells were counted and percentage of IF-positive cells was calculated on 3, 5, and 7 days after infection. Control (●——●), 100 J/m^2 (▲——▲), 200 J/m^2 (▲– – –▲), 500 J/m^2 (▲–·–▲), 1000 J/m^2 (△——△), 2000 J/m^2 (△– – –△), and 5000 J/m^2 (△–·–△) UV-irradiated HTLV-III infected MT-4 and uninfected MT-4 (○——○).

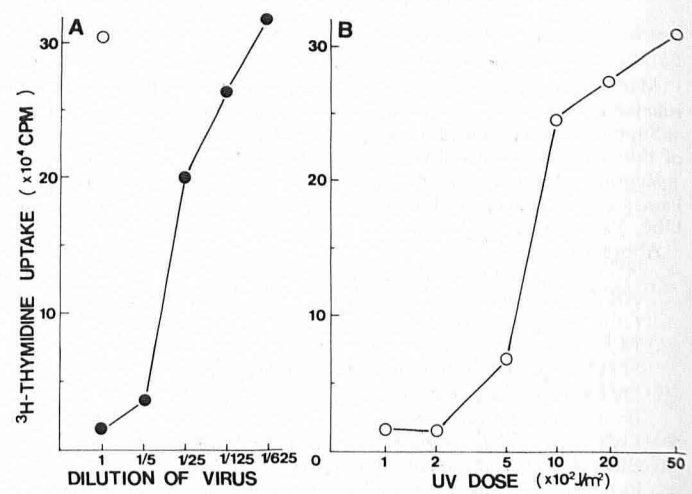

Figure 3. Effect of UV irradiation on the virus infectivity (*B*) detected by [^{3}H]thymidine uptake method. *A*, viral dose-response curve. Dilution 1 of the virus was used for the inactivation experiment in (*B*). Incorporation of [^{3}H]thymidine in infected MT-4 was determined 5 days after infection. *A*, Uninfected MT-4 (*open circles*), HTLV-III-infected MT-4 (*solid circles*).

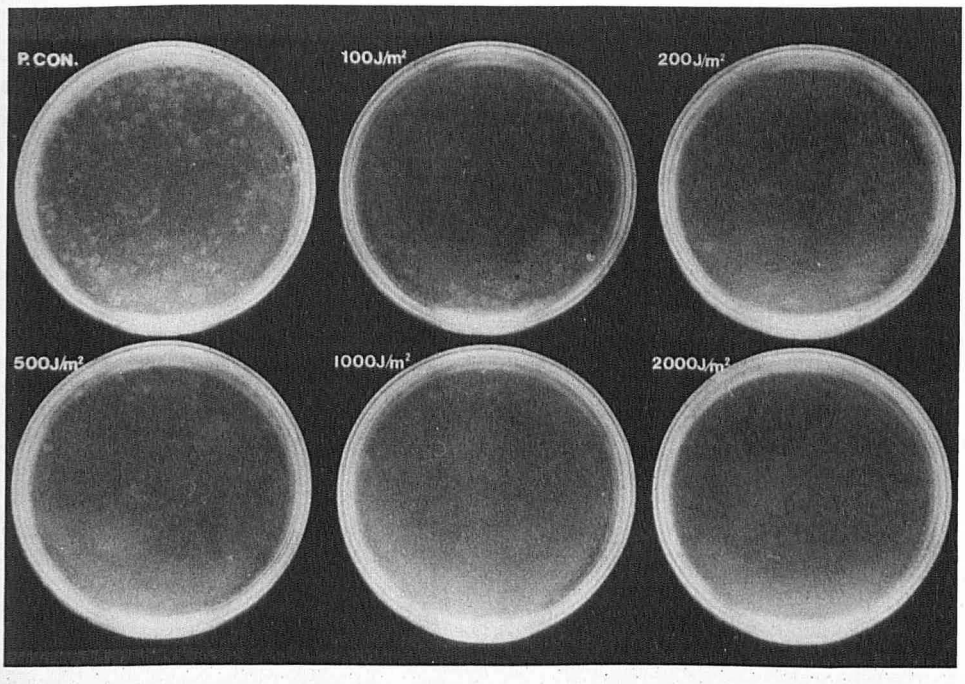

Figure 4. Plaque-forming assay with UV-irradiated and unirradiated control HTLV-III in MT-4 cells. The assay was performed in triplicate.

infected MT-4 cells (Fig 1). The number of IF-positive cells expresses the amount of biologically active virus [17]. On the 7th day after infection, about 90% of the IF-positive cells were detectable in both unirradiated and 100 J/m^2 UV-irradiated HTLV-III-infected MT-4 cells. At this point in time, 67, 59, and 49% of IF-positive cells were detected in MT-4 cells infected by 200 J/m^2, 500 J/m^2, and 1000 J/m^2 UV-irradiated viruses, respectively. Moreover, 2000 J/m^2 UV-irradiated virus-infected MT-4 cells showed only 4% of the IF-positive cells and 5000 J/m^2 UV-irradiated virus-infected cells showed absolutely no IF-positive cells.

Virus-induced cell damage was determined by the trypan blue dye exclusion method. On the 7th day after infection, nearly 90% of infected cells were dead when HTLV-III was treated by 100 J/m^2 of UV irradiation. However, cell damage was lower than 10% in 2000 and 5000 J/m^2 UV-irradiated virus-infected MT-4 cells as well as uninfected cells (Fig 2).

Inhibition of UV-Irradiated Virus on DNA Synthesis in MT-4 Cells To further confirm the inactivation of the virus by UV irradiation, a proliferation assay was performed. HTLV-III-induced CPE is linked to the appearance of viral antigens which are followed by the inhibition of DNA synthesis in HTLV-III-infected MT-4 cells [14]. Fig 3*A* demonstrates that uninfected MT-4 cells showed over 300,000 cpm of [^{3}H]thymidine incorporation, whereas only 10,000 cpm appeared in HTLV-III-infected MT-4 cells on the 5th day after infection. The inhibition of cellular DNA synthesis by UV-irradiated HTLV-III in MT-4 cells is displayed in Fig 3*B*. [^{3}H]Thymidine uptake was inhibited in MT-4 cells infected by HTLV-III irradiated, respectively, with 100 J/m^2, 200 J/m^2, and 500 J/m^2 UV, whereas its inhibition was very weak in MT-4 cells infected by 1000 J/m^2 and 2000 J/m^2 UV-irradiated virus and practically no inhibition was recognized in the cells infected by 5000 J/m^2 UV-irradiated virus.

Inactivation of HTLV-III by UV Irradiation Detected by Plaque-Forming Assay To attest the inactivation of HTLV-III by UV irradiation more distinctly, plaque-forming assay was performed (Fig 4, Table I). Although 100 J/m^2 UV-irradiated virus did not show a significant difference compared with unirradiated HTLV-III, 2000 J/m^2 UV-irradiated HTLV-III showed only 1 plaque in 3 dishes and 5000 J/m^2 UV-irradiated virus showed no plaques.

Morphologic Change of the MT-4 Cells Infected by UV-Irradiated HTLV-III Morphologic change of MT-4 cells infected by UV-irradiated HTLV-III was observed as the degeneration of ballooning which was a CPE rarely seen in MT-4 cells infected by unirradiated HTLV-III after 10 days of culture. Cloned HTLV-III (UV-1) was obtained from a plaque which was induced by 2000 J/m^2 UV-irradiated HTLV-III. The clone UV-1 also induced ballooning in infected MT-4 cells (Fig 5*A*) and this change was not shown in MT-4 cells infected by unirradiated HTLV-III (Fig 5*B*). These ballooning cells showed HTLV-III viral-specific antigen which was indistinguishable from unirradiated HTLV-III (Fig 5*C,D*).

Resistance to UV of the Cloned HTLV-III Isolated After UV Irradiation To investigate whether the clone UV-1 has more UV resistance than unirradiated HTLV-III, plaque-forming assay was performed in the same manner. As shown in Table II, the resistance to UV irradiation was not different between clone UV-1 and unirradiated HTLV-III.

DISCUSSION

Since the initial reports of AIDS were made in 1981 [19,20] the number of cases reported each year has increased dramatically. Characteristically, AIDS is associated with a progressive depression of T cells, especially the helper/inducer subset bearing the OKT-4 surface marker [21]. It is plausible that the immunodeficiency status of patients was induced by HTLV-III infection in

Table I. Effects of UV Irradiation on HTLV-III Infectivity Detected by Plaque Formation in MT-4 Cells

UV Dose (J/m^2)	Number of Plaques per Dish
Control	105.3 ± 6.0[a]
100	92.7 ± 9.0
200	55.3 ± 3.6
500	12.0 ± 0.7
1000	2.0 ± 0.7
2000	0.3 ± 0.6
5000	0

[a]Experiments were carried out in triplicate. Number represents the mean ± SD.

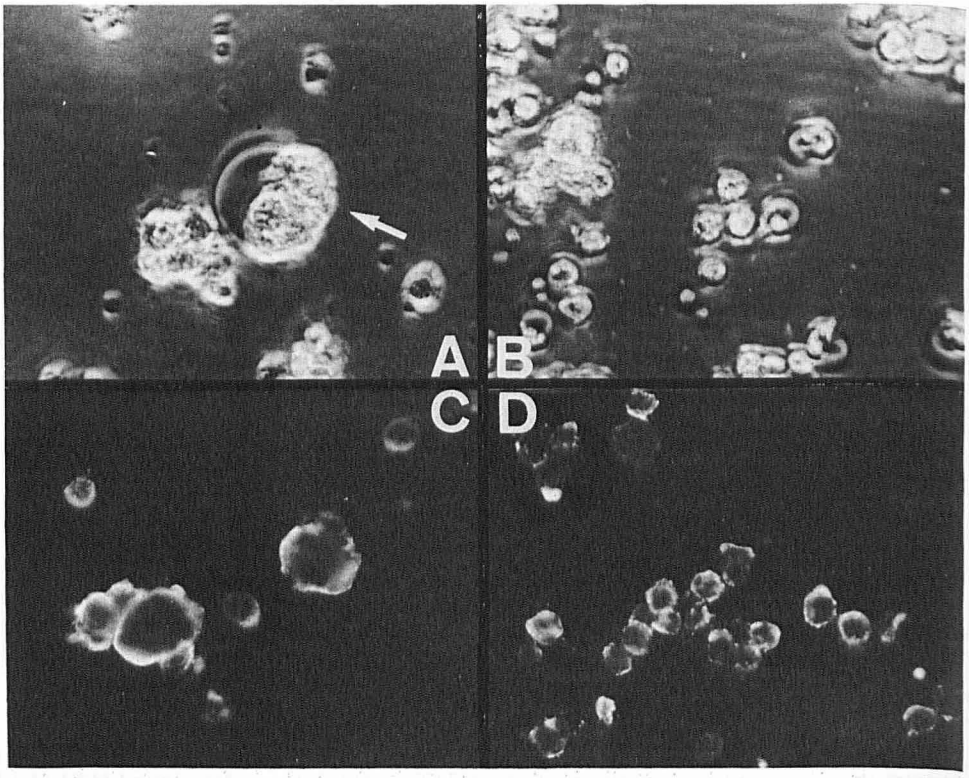

Figure 5. Morphologic change and IF-positive cells in MT-4 infected by clone UV-1 and unirradiated HTLV-III/LAV. The clone UV-1 was obtained from a plaque formed by 2000 J/m^2 UV-irradiated HTLV-III. The clone UV-1 induced ballooning degeneration in MT-4 cells (*A*, *arrow*), however these ballooning cells were not seen in MT-4 cells infected by unirradiated HTLV-III (*B*). These ballooning cells showed the same HTLV-III-specific antigens (*C*) as detected in MT-4 cells infected by unirradiated HTLV-III (*D*).

vivo. Also, the virus was thought to be spread among patients with hemophilia through the injection of factor VIII concentrate. However, an effective therapy has not been found to consistently treat immunedeficiency in AIDS. Thus, it is urgent to develop clinical or biologic methods not only for cure but also for inactivation of the virus itself.

Spire et al [13] reported inactivation of LAV by heat, γ-rays, and UV light by measuring reverse transcriptase (RT) activity. However, RT activity does not represent the amount of the virus, especially in terms of biologic activity of the virus. In view of the fact that even a single infectious virion can multiply to several magnitudes and increase the amount of virus in the infected cells if the appropriate circumstances are provided, it is most important to have a way to measure residual viral activity accurately after the physical or chemical treatment of the blood products. Recently, we also reported that HTLV-III was inactivated by heating to 56°C for 30 min, using quantitative methods such as proliferation assay and the virus-specific antigen induction of the infected MT-4 cells [14]. In the present study, we have attempted to evaluate the effect of UV irradiation on the infectivity of HTLV-III by these bioassay methods.

Our results indicate that UV has a dose-dependent inhibitory effect on HTLV-III infection in MT-4 cells and infectivity of HTLV-III was completely abolished by 5000 J/m^2 of UV irradiation. The results reported (Fig 1) were limited to measuring, at the most a 2 log kill of the AIDS virus. Moreover, Fig 3 shows that at least 1/625 of the virus was also killed by the 5000 J/m^2 of UV. Our data roughly match a previous report by Spire et al [13]. These results suggest that UV irradiation is a simple and useful method to inactivate HTLV-III.

Furthermore, we noticed that 2000 J/m^2 UV-irradiated HTLV-III, clone UV-1, induced quite an interesting morphologic change which was very rarely seen when MT-4 cells were infected by a control virus. Two possibilities about this UV-irradiated HTLV-III-induced morphologic change were considered: (1) UV irradiation induced the mutation of the virus itself. It is well known that UV and other kinds of radioactivity induce nucleic acid degeneration and/or mutation of viruses [22]. (2) UV irradiation simply selected a cloned virus with such unusual activity, since HTLV-III were isolated from the blood pool of several AIDS patients and genomic diversity between HTLV-III isolates was noted [23,24]. If UV irradiation might generate mutants of HTLV-III, an alternative sterilization method which is lethal but not mutagenic may be preferable, especially for blood products. However, UV irradiation is still a simple and useful method to inactivate HTLV-III/LAV.

Finally, blood products are now screened by enzyme-linked immunosorbent assay (ELISA) to detect antibodies against HTLV-III/LAV. However, it remains important to discover means for inactivation of HTLV-III/LAV before they are administered to patients, because some patients or individuals have no antibody to HTLV-III/LAV but have the virus in their sera [25,26]. Moreover, our bioassay system should be useful in future studies to quantitate the effects of other inactivating agents or antiviral drugs on HTLV-III/LAV.

Table II. Comparison of UV Sensitivity Between HTLV-III/LAV and Clone UV-1 Detected by Plaque Formation

UV Dose (J/m^2)	Number of Plaques per Dish	
	HTLV-III	Clone UV-1
Control	49.0 ± 12.0[a]	23.0 ± 2.0[a]
500	11.0 ± 5.2	3.7 ± 2.1
1000	3.0 ± 0	2.3 ± 0.6
2000	0.7 ± 1.2	2.3 ± 1.2

[a]Experiments were carried out in triplicate. Number represents the mean ± SD.

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